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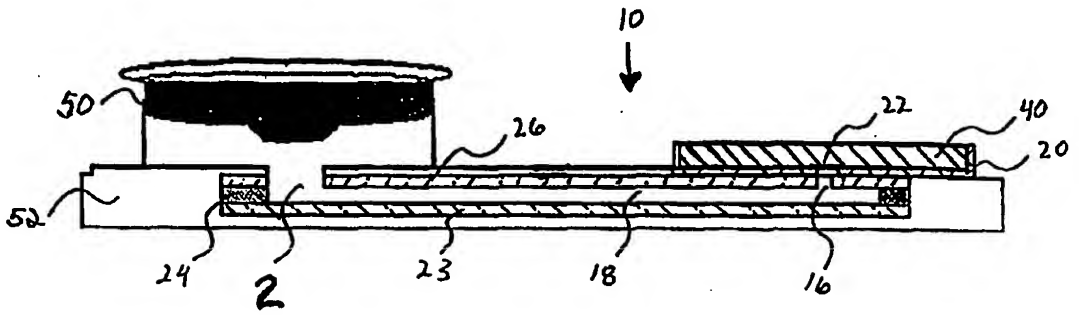

(57) Abstract

The present invention is directed to improving the performance of assays using a disposable assay device which includes a porous material (20) in liquid communication with a capillary track (18). In particular, the capillary track (18) is used in conjunction with the solid support to direct test sample and assay reagents directly to a defined reaction site (22) on or in the porous material (20). Signal development at the reaction site (22) indicates the assay result. The present invention is also directed to the construction of a disposable assay device (10) which includes a capillary track (18). In particular, the capillary track is formed by printing a fluid insoluble material (25a), in the reverse image of the desired capillary track (18), on a film layer (23) and then capping the printed material. Alternatively, the capillary track (18) is formed by printing a fluid insoluble material, in the reverse image (30) of the desired capillary track (18), on a porous material (25b) which is then sandwiched between two film layers (23, 26). The present invention is also directed to a drop-forming means (50) which may incorporate one or more assay reagents to allow for improved sample processing and reagent addition, mixing and incubation.

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DEVICES HAVING SUBSURFACE FLOW AND THEIR USE IN DIAGNOSTIC ASSAYS

BACKGROUND OF THE INVENTION

This patent application is a continuation-in-part of US Serial No. 07/958,068, filed October 8, 1992, now abandoned.

1. Field of the Invention

In general, the present invention relates to assay methods and devices for the detection of an analyte in a test sample. In particular, the invention relates to novel test devices designed to provide for the rapid transfer of fluid to a reactive membrane by means of a capillary track. In addition, the invention relates to novel methods for forming a capillary track and novel sample processing devices for use in diagnostic testing.

2. Related Art

Various analytical procedures and devices are commonly employed in assays to determine the presence and/or amount of substances of interest which may be present in biological or non-biological fluids. Such substances are commonly termed "analytes". The ability to use materials which specifically bind to an analyte of interest has created a burgeoning diagnostic device market based on the use of binding assays.

Binding assays incorporate specific binding members, typified by antibody and antigen immunoreactants, wherein one member of the specific binding pair is labeled with a signal-producing compound. For example, in a binding assay the test sample suspected of containing analyte can be mixed with a labeled anti-analyte antibody, i.e., labeled reagent, and incubated for a period of time sufficient for the immunoreaction to occur. The reaction mixture is subsequently analyzed to detect either that label which is associated with an analyte/labeled reagent complex (bound labeled reagent) or that label which is not complexed with analyte (free labeled reagent). As a result, the presence or amount of label in one of these species can be correlated to the presence or amount of analyte in the test sample.

The solid phase assay format is a commonly used binding assay technique. There are a number of assay devices and procedures wherein the presence of an analyte is indicated by the binding of the analyte to an labeled reagent and/or an immobilized complementary binding member. The immobilized binding member is bound, or becomes bound during the assay, to a solid phase such as a dipstick, teststrip, flow-through pad, paper, fiber matrix or other suitable solid phase material. The binding reaction between the analyte and/or assay reagent(s) results in a distribution of the labeled reagent between that which is immobilized upon the solid phase and that which remains free. The presence or amount of analyte in the test

sample is typically indicated by the extent to which the labeled reagent becomes immobilized upon the solid phase material.

Flow-through pads for immobilizing and detecting an analyte are well-known in the art. For example, Tom et al. (United States Patent No. 4,366,241) disclose a bibulous strip with an immunosorbing zone to which the test sample is directly applied and wherein the assay result is detected.

The use of reagent-impregnated teststrips in specific binding assays is also well-known. In such procedures, a test sample is applied to one portion of the teststrip and is allowed to migrate or wick through the strip. Thus, the analyte to be detected or measured passes through or along the strip, possibly with the aid of an eluting solvent which can be the test sample itself or a separately added solution. The analyte migrates into a capture or detection zone on the teststrip, wherein a complementary binding member to the analyte or labeled reagent has been immobilized. The extent to which the analyte becomes bound in the detection zone can be determined with the aid of the labeled reagent which can also be incorporated in the teststrip or which can be applied separately.

An early teststrip device is described by Deutsch et al. in United States Patent No. 4,361,537. In general, the device comprises a material capable of transporting a solution by capillary action, i.e., a wicking or chromatographic action. Different areas or zones in the teststrip contain the assay reagents needed to produce a detectable signal as the analyte is transported to or through such zones. The device is suited for both chemical assays and binding assays and uses a developer solution to transport analyte along the strip.

The disadvantages of the conventional porous or absorbent matrix devices include the slow rate of flow of test sample through the teststrip material. In addition, the test sample and mobile reagents are directed to and through the edge of an absorbent pad or layer containing the reaction site. Such diffuse sample application results in reduced signal production and a slowed rate of signal production.

Assay devices have also been constructed of tubes wherein the capillary tube contains an immobilized assay reagent to define a reaction zone for the capture and detection of an analyte of interest (Hibino et al., 4,690,907). In general, the capillary tube is used to collect a predetermined amount of test sample for use in a test device.

Conventional capillary tracks are formed from glass tubes. Glass tubes, however, are usually restricted to simple geometric designs. Glass tracks are also breakable and a biosafety hazard to workers. Other typical capillary tracks are constructed by sandwiching a die-cut material between two pieces of film, wherein one film is typically more hydrophobic than the other for the purpose of promoting

fluid movement. This type of device is limited to simple single track designs because of manufacturing limitations involving the placement of the die-cut middle layer.

In still another conventional design, capillary tracks are formed through a process of injection molding. A major disadvantage of this process is the cost of prototyping. Another disadvantage is in the manufacturing process which is limited to piece-part assembly. Also, the use of multiple materials complicates construction and assembly. When dissimilar materials are incorporated as different layers, the separate pieces must be spot treated during assembly. For example, a sandwich layer of adhesive would be needed to mate the pieces together. Alternatively, the materials could be ultrasonically welded or solvent bonded, but the manufacturing limitations remain.

SUMMARY OF THE INVENTION

The present invention is directed to improving the performance of assays using a disposable assay device which includes a porous material in liquid communication with a capillary track. In particular, the capillary track is used in conjunction with the solid support to direct test sample and assay reagents directly to a defined reaction site on or in the porous material. Signal development at the reaction site indicates the assay result.

The present invention is also well suited for enhancing the production of signal at the reaction site. The capillary track directs fluid to a position below the defined reaction site on the porous material such that the fluid does not have to pass through the edge of the porous material, as in conventional teststrip device, to reach the reaction site. Preferably, the test sample is directed to a position directly below the reaction site on the porous material. Upon contact with the porous material, the fluid passes radially through the reaction site rather than transversely through the site.

The devices of the present invention are constructed from a capillary track having an inlet and outlet, wherein the inlet receives test sample or test solution, and the outlet is in communication with and directs test sample or test solution to a porous support containing an immobilized reagent which binds to the analyte, an ancillary specific binding member or a labeled reagent to produce a detectable assay result. The outlet port is disposed beneath the immobilized reagent in the porous support. The device may further include a labeled reagent such that the reagent need not be separately contacted to the device and such that the assay method is self-performing. The labeled reagent may be contained within the capillary track or within a material or means which is in fluid communication with the capillary track. In a preferred embodiment, a reagent matrix is contained within a drop forming means which is in communication with the capillary track. Assay kits are also

contemplated and include the subsurface flow device together with one or more containers of reagents necessary to the performance of the assay.

The present invention is also directed to a drop-forming means which incorporates one or more assay reagents in a single or multiple-layer chamber to allow for improved sample processing and reagent addition, mixing, and incubation. The drop-forming means may be a component of the assay device or, alternatively, may be a free-standing component which is placed over the inlet of the device of the invention or over the sample application zone of prior art assay devices. A preferred embodiment has the drop-forming means affixed to the inlet of a capillary-track device of the present invention. Application of sample fluid to the drop-former allows mixing and incubation of sample and reagents within the matrix of the drop-former. After passing through the drop-forming means, sample is delivered to the inlet of the capillary track.

The present invention is also directed to constructing a disposable assay device which includes a capillary track. One method for constructing the capillary track involves applying a printable material to a first film layer thereby forming a core layer and three sides of the capillary track, wherein the printable material is deposited as a reverse image of the capillary track on the first film layer. A second film layer is then adhered to the top of the printable material or core layer, thereby forming the fourth side of the capillary track.

An alternative method for constructing the capillary track involves applying a fluid repellent printable substance to a length of porous material thereby impregnating the porous material wherein a non-impregnated region defines two sides of the capillary track. A first and a second film layer are then adhered to the top and bottom of the porous material thereby forming the top and bottom of the capillary track.

The present invention can also be adapted for use in the automated diagnosis of a plurality of samples. Another object of the present invention is to provide a device capable of performing multiple, highly sensitive, diagnostic tests simultaneously on a single sample in a single device having multiple capillary tracks and reaction sites. In particular, the devices of the present invention can be used in an automated fashion where the assay reaction can be rapidly performed and monitored with a minimum of sample material.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a side perspective of one embodiment of the present reaction device.

Figure 2 is a side perspective of the present reaction device showing two layer construction.

Figure 3 is a side perspective of the present reaction device showing three layer construction.

Figure 3a depicts a modified embodiment of the present invention.

Figure 3b is a side perspective of a multi-layer drop-forming means.

Figure 4 is an end view of a reaction device showing a printed layer construction.

Figure 5 is an end view of a reaction device showing a multi-layer construction using a partially impregnated porous material as the middle layer.

Figure 5a is an end view of a reaction device of the invention showing a multi-layer construction using a partially impregnated porous material as the middle layer which is an adhesive.

Figure 6 is an end view of a reaction device showing a three layer construction.

Figure 6a is a side perspective of an enhanced assay device having multiple islands of non-impregnated porous material.

Figure 7 is a top perspective of an enhanced assay device having directed flow through the reaction site.

Figure 8 is side perspective of an enhanced assay device having an absorbent layer.

Figure 8a is a side perspective of an enhanced multi-layer reaction device having a drop-forming means.

Figure 9 depicts predicted chromatographic flow rates in a linear and radial flow format.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Before proceeding with the description of the various embodiments of the present invention, a number of terms used herein will be defined.

"Test sample" refers to a material suspected of containing the analyte. The test sample can be used directly as obtained from the source or after pretreatment so as to modify its character. The test sample is typically a physiological fluid. The test sample can be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, extracting analyte, or the like. Methods of treatment can involve filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples can be used such as water, food products and the like for the performance of environmental or food production assays as well as diagnostic assays. In addition, a solid material suspected of containing the analyte can be used as the test sample once it is modified to form a liquid medium or to release the analyte.

"Specific binding member" refers to a member of a specific binding pair, i.e., two different molecules wherein one of the molecules specifically binds to the second molecule through chemical or physical means. In addition to antigen and antibody specific binding pair members, other exemplary specific binding pairs include, without limitation, such materials as biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, complementary peptide sequences, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, a peptide sequence and an antibody specific for the sequence or the entire protein, polymeric acids and bases, dyes and protein binders, peptides and specific protein binders (e.g., ribonuclease, S-peptide and ribonuclease S-protein). Furthermore, specific binding pairs can include members that are analogs of the original specific binding member, for example an analyte-analog or a specific binding member made by recombinant techniques or molecular engineering. If the specific binding member is an immunoreactant it can be, for example, an antibody, antigen, hapten, or complex thereof, and if an antibody is used, it can be a monoclonal or polyclonal antibody, a recombinant protein or antibody, a chimeric antibody, a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific binding members are well-known to those skilled-in-the-art.

"Analyte" or "analyte of interest" refers to the compound or composition to be detected or measured, which has at least one epitope or binding site. The analyte can be any substance for which there exists a naturally occurring analyte-specific binding member or for which an analyte-specific binding member can be prepared. Analytes include, but are not limited to toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), and metabolites of or antibodies to any of the above substances. The term "analyte" also includes any antigenic substances, haptens, antibodies, macromolecules and combinations thereof.

"Analyte-analog" refers to a substance which cross-reacts with the analyte-specific binding member, although it may do so to a greater or a lesser extent than does the analyte itself. The analyte-analog can include a modified analyte as well as a fragmented or synthetic portion of the analyte molecule, so long as the analyte-analog has at least one epitopic site in common with the analyte of interest. An example of an analyte-analog is a synthetic peptide sequence which duplicates at least one epitope of the whole-molecule analyte so that the analyte-analog can bind to an analyte-specific binding member.

"Labeled reagent" refers to a substance comprising a detectable label attached to a specific binding member. The attachment may be covalent or non-covalent

binding, direct or indirect, but the method of attachment is not critical to the present invention. The label allows the labeled reagent to produce a detectable signal that is directly or inversely related to the amount of analyte in the test sample. The specific binding member component of the labeled reagent may be selected to directly bind to the analyte or to indirectly bind the analyte by means of an ancillary specific binding member, which is described in greater detail hereinafter. Alternatively, the specific binding member component may be selected to directly or indirectly bind an immobilized reagent. The labeled reagent can be incorporated into the test device, it can be combined with the test sample to form a test solution, it can be added to the device separately from the test sample or it can be predeposited or reversibly immobilized at the immobilized reagent site. In addition, the binding member may be labeled before or during the performance of the assay by means of a suitable attachment method.

"Label" refers to any substance which is capable of producing a signal that is detectable by visual or instrumental means. Various labels suitable for use in the present invention include labels which produce signals through either chemical or physical means. Such labels can include enzymes; enzyme substrates; chromogens; catalysts; fluorescent compounds; chemiluminescent compounds; radioactive labels; and direct visual labels including colloidal metallic particles such as gold, colloidal non-metallic particles such as selenium, dyed or colored particles such as a dyed plastic or a stained microorganism, organic polymer latex particles and liposomes or other vesicles containing directly visible substances. A visually detectable label is advantageously used as the label component of the labeled reagent, thereby providing for the direct visual or instrumental readout of the presence or amount of the analyte in the test sample without the need for additional signal producing components at the detection sites.

The selection of a particular label is not critical to the present invention, but the label will be capable of generating a detectable signal either by itself, such as a visually detectable colored organic polymer latex particle, or be instrumentally detectable, such as a fluorescent compound. The label may be detected in conjunction with one or more additional signal producing components, such as an enzyme/substrate signal producing system. A variety of different labeled reagents can be formed by varying either the label component or the specific binding member component of the labeled reagent; it will be appreciated by one skilled-in-the-art that the choice involves consideration of the analyte to be detected and the desired means of detection.

"Signal producing component" refers to any substance capable of reacting with another assay reagent or with the analyte to produce a reaction product or signal that indicates the presence of the analyte and that is detectable by visual or instrumental

means. "Signal production system", as used herein, refers to the group of assay reagents that are needed to produce the desired reaction product or signal. One or more signal producing components can be reacted with the label to generate a detectable signal. For example, when the label is an enzyme, amplification of the detectable signal is obtained by reacting the enzyme with one or more substrates or additional enzymes and substrates to produce a detectable reaction product.

"Porous support" refers to any suitable porous, absorbent, bibulous, isotropic or capillary material, which includes the reaction site of the device. Natural, synthetic, or naturally occurring materials that are synthetically modified, can be used as the porous support and include, but are not limited to: papers (fibrous) or membranes (microporous) of cellulose materials such as paper, cellulose, and cellulose derivatives such as cellulose acetate and nitrocellulose; fiberglass; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; porous fibrous matrixes; starch based materials, such as cross-linked dextran chains; ceramic materials; olefin or thermoplastic materials including films of polyvinyl chloride, polyethylene, polyvinyl acetate, polyamide, polycarbonate, polystyrene, copolymers of vinyl acetate and vinyl chloride and combinations of polyvinyl chloride-silica; and the like. The porous material should not interfere with the production of a detectable signal.

"Immobilized reagent" refers to a specific binding member that is attached within or upon a portion of the porous support to form a "capture site" or reactive membrane. The method of attachment is not critical to the present invention. The extent of signal production in the capture site is related to the amount of analyte in the test sample. The immobilized reagent is selected to bind the analyte, the labeled reagent or a complex thereof. In preferred embodiments, the immobilized reagent binds to the analyte for the completion of a sandwich complex. Competitive assay formats will also be apparent to those skilled-in-the-art. The immobilized reagent may be chosen to directly bind the analyte or indirectly bind the analyte by means of an ancillary specific binding member which itself is bound to the analyte. In addition, the immobilized reagent may be directly or indirectly immobilized on the solid phase before or during the performance of the assay by means of any suitable attachment method.

Typically, the capture site of the present invention is a delimited or defined portion of the porous support such that the specific binding reaction of the immobilized reagent and analyte is localized or concentrated in a delimited site. Such a localization facilitates the detection of label that is immobilized at the capture site in contrast to other portions of the porous support. The delimited site is typically less than 50% of the porous support, and preferably less than 25% of the porous support. The immobilized reagent can be applied to the solid phase material by

dipping, inscribing with a pen, dispensing through a capillary tube or through the use of reagent jet-printing or any other suitable dispensing techniques. In addition, the capture site can be marked, for example with a dye, such that the position of the capture site upon the porous support can be visually or instrumentally determined even when there is no label immobilized at the site.

Predetermined amounts of signal producing components and ancillary reagents can be incorporated within the device, thereby avoiding the need for additional protocol steps or reagent additions. Thus, it is also within the scope of this invention to provide more than one reagent to be immobilized within the porous support. For example, to slow or prevent the diffusion of the detectable reaction product in an enzyme/substrate signal producing system, the substrate can be immobilized by direct attachment to the porous support by methods well-known in the art, or the substrate may be immobilized by being covalently bound to insoluble microparticles which have been deposited in and/or on the porous support.

The immobilized reagent may be provided in a single capture or detection site or in multiple sites on or in the porous support. The immobilized reagent may also be provided in a variety of configurations to produce different detection or measurement formats. Alternatively, the immobilized reagent can be distributed over a large portion of the porous support in a substantially uniform manner to form the capture site.

"Ancillary specific binding member" refers to any member of a specific binding pair which is used in the assay in addition to the specific binding members of the labeled reagent or immobilized reagent. One or more ancillary specific binding members can be used in an assay. For example, an ancillary specific binding member may be used to bind the labeled reagent to the analyte in instances where the analyte itself could not directly bind the labeled reagent. The ancillary specific binding member can be incorporated into the assay device or it can be added to the device as a separate reagent solution.

One exemplary embodiment of the present invention is shown in Figure 1. The device (10) includes a main body (12) in which a capillary track (18) extends along at least a portion of its length. The capillary track has a size and dimensions suitable for the transport of test sample through the track by capillary action. The capillary track has an inlet (14) for the introduction of test sample to the device. The capillary track is in fluid communication with a porous support (20) containing an immobilized specific binding material (22). The capillary track has an outlet (16) in fluid flow communication with the porous support. Preferably, the porous support is positioned such that the outlet of the capillary track lies directly beneath the site of the immobilized specific binding material. For purposes of the present invention, "directly beneath" means that fluid passing from the capillary track to the porous

support passes through one-half or less of the largest dimension of the immobilized reagent (capture site) on the porous support. More preferably, fluid passing from the capillary track to the porous support passes through less than one-quarter, most preferably one-tenth or less, of the largest dimension of the immobilized reagent on the porous support.

Figure 1 depicts a device wherein the capillary track outlet is in direct contact with the porous support and is positioned beneath the reaction site of immobilized specific binding material. It is not essential to the present invention that the outlet and porous support be in direct contact. It is sufficient that the components are close enough that fluid in the capillary track will pass from the outlet to the porous support. Nor is it essential that the outlet be positioned directly under the reaction site. It will be readily appreciated, however, that the closer the capillary track outlet is to the reaction site, the less distance the test sample and/or assay reagents must travel.

Assay reagents, such as a labeled specific binding member may be, mixed with the test sample, sequentially contacted to the test device, incorporated into a drop-forming means, or included within the capillary track. For example, the labeled reagent may be predeposited in the capillary track such that contact with the test sample mobilizes the labeled reagent and transports the labeled reagent to the reaction site on the porous support. This is to be distinguished from agglutination assay devices wherein the reaction of assay reagents and test sample analyte results in the formation of a reaction product which agglutinates and decreases or stops fluid flow within a capillary space.

A second embodiment of the present invention is shown in Figure 2. The device (10) generally comprises a main body (12) constructed from a first surface (6) and a parallel second surface (8) one or both of which are grooved, separated by spacers or otherwise constructed such that when the two surfaces are aligned and joined a capillary track (18) is formed. The capillary track has a size and dimensions suitable for the transport of test sample and soluble assay reagents through the track by capillary action. The surfaces may be joined by any suitable means including, but not limited to, sonic welding, solvent welding and adhesive bonding. In the adhesive bonding method, the adhesive may be applied by a printing means.

Figure 3 represents a further embodiment of the present invention. This embodiment has, in adherent relationship, a first or bottom wettable, but liquid-occlusive, layer (**23**), a second or middle liquid occlusive layer (24) parallel to and overlying the first layer (**23**), and a third or top liquid-occlusive, preferably non-wettable, layer (26) parallel to and overlying the second layer (24). The third layer (26) may be made from a clear material, such as a clear polycarbonate film, and therefore, the layer may also serve as a window, or viewing area, for observing

the capillary track. The second layer (24) is interposed between, and is adhered to, the first layer (23) and third layer (26). For example, the layers are adhered by means of an adhesive on each side of the second layer (24) facing the topside of the first layer (23) and the underside of the third layer (26). Typically, the second layer (24) is die cut or preformed to have a slot positioned through its thickness, thereby defining the walls of the capillary track (18) in conjunction with the first (23) and third (26) layers. Thus, when the first, second and third layers are laminated together, a portion of each of the first and third layers serve as the floor and roof, respectively, of the capillary track with part of the walls of the slot of the second layer (24) defining the walls of the capillary track.

The device illustrated in Figure 3 may also include an optional well-defining means (2) in the third layer (26). The well-defining means is positioned such that the it defines an area for receiving the test sample, and it is in fluid flow communication with the capillary track. The bottom of the well may be formed from a corresponding circular portion of the first layer (23).

In a preferred embodiment, the devices of the present invention include a test sample application pad in fluid flow contact with the capillary track. The application pad facilitates the application of test sample or reagents to the device and may optionally contain one or more reagents, such as the labeled binding member. The addition of test sample to the application pad serves to elute an assay reagent from the application pad, such that a test sample/reagent mixture emerges from the bottom surface of the application pad. The device may further include a well situated between the application pad and the capillary track inlet such that the test solution exiting the application pad substantially fills the well prior to passing into the capillary track. The application pad may be constructed from a single material or from a plurality of layers. The use of a multi-layered application pad permits the inclusion of multiple assay reagents, even when the reagents are not compatible for extended storage, thereby allowing multiple, separate reagent additions to the test sample.

The application pad material or a layer thereof may also be selected to provide a filtering function. In this case, the filter can function as a sample "prefilter" to remove particulate from the test sample so that particulate does not clog or alter flow in the capillary track or the porous support. For urine test samples the particulate can vary from patient to patient, with the typical range being from the submicron (<1μ) to about 50μ. The total amount and percentage present within a particular range will also vary from patient to patient. When a prefilter is used in conjunction with the preferred porous support (5μ nitrocellulose) of the present invention it is of course necessary to select a prefilter which functions to remove particulates which are larger than 3 to 5μ to prevent clogging of the nitrocellulose membrane. Furthermore, more than one prefilter may be used to provide additional particulate

retention capacity. A number of suitable materials are available commercially and the particulate cutoff can be varied depending on the prefilter used and as may be required for the assay format employed. For example, materials which provide acceptable prefiltering of urine in an assay for human chorionic gonadotropin (hCG), include: Lydall® Grade 254 (Lydall, Rochester, NH, USA); Nalgene® 281-9000 (Nalge Inc., Rochester, NY, USA); F300-05 and F366-02 (Whatman, Inc., Maidstone, Kent, Great Britain); DD 2391 and DE 1381 (Hollingsworth & Vose, West Groton, MA, USA); Tetko 3 (Tetko, Elmsford, NY, USA); and Pall Loprodyne 5μ (Pall, East Hills, NY, USA). The preferred prefilter in this assay format is Lydall® Grade 254 in combination with Nalgene® 281-9000.

Figure 3a depicts an alternative embodiment wherein the device (10) includes a drop-forming means (50) which holds an application pad (55) that contains the labeled reagent. By contacting a test sample to the drop-forming means, the labeled reagent is released from the application pad and forms a drop on the bottom surface of the application pad. The drop-forming means is situated over the capillary track such that when the drop is released from the application pad it is delivered to the capillary track. This optional modification provides an added advantage. The addition of fluid or test sample to the reagent-containing application pad serves to deliver a bolus of the eluted reagent to the capillary track. Thus, the first fluid mixture entering the capillary track contains a large portion of the eluted reagent, and subsequent fluid contains less of the reagent. When the reagent is a labeled reagent, this means that the first fluid delivered to the reaction site on the porous support contains the largest portion of the labeled reagent. Subsequent fluid contains a lesser amount of assay reagent and thereby enhances the clearance of unreacted reagents from the reaction site. This clearance or washing aspect of the invention helps to stabilize the signal that is produced in the reaction site and decreases interference from the occurrence of background signal in the area surrounding the reaction site.

In yet a further embodiment of the invention, as depicted in Figure 3b, the device includes a drop-forming means (50) which holds a multi-layered application pad (56a-c). The multi-layered application pad is constructed from a plurality of porous layers to allow the inclusion of multiple assay reagents, to enhance sensitivity, to prevent erroneous signal development (false negatives or false positives), to enhance mixing of test sample and reagents within the drop-former, to allow increasd incubation time to promote reaction efficiency, to increase signal intensity, etc. The drop-former may be affixed adjacent the inlet port or, alternatively, may be configured to be removable which allows the device to be easily and rapidly reconfigured for differing assay requirements. As described above, a prefilter may be employed in the drop-former means to prevent clogging or altered flow in the capillary track or at the porous support or which may interfere with the

accuracy of the assay results. As will be apparent to the skilled person, the number of layers and the composition of each layer can be adjusted to suit the differing reagent requirements of the assay to be performed or the condition of the sample to be analyzed. In a preferred arrangement, a labeled reagent may be incorporated into one of the pad materials such that the labeled reagent incubates with the sample prior to the test sample being delivered to the capillary track and thereby allowing for an intensified signal. Additional embodiments can, for example, utilize multiple layers to allow for cell separation from whole blood, to allow the use of serum, urine, or plasma samples, to detect more than one analyte in a test sample, or to allow the use of different labelled reagents for assay control purposes.

As shown in Figure 3b, the multi-layered application pad (56) includes an upper layer (56a), a middle layer (56b) and a bottom layer (56c). In a preferred embodiment, the upper layer is a prefilter constructed of glass fiber, most preferably Nalgene #281-9000, the middle layer contains the labeled reagent in a glass fiber pad, most preferably Lydall glass fiber, and the bottom layer is preferably composed of absorbent paper (cellulose).

The use of a drop-former as described above but as a separate, or free-standing component is also contemplated as being within the scope of the invention. In this aspect the drop-forming means can be placed adjacent to, for example over or above, the sample application area or zone of a teststrip device and the drop delivered to the strip. In this way the advantages of the drop-forming means, i.e. sample mixing, delivery of a bolus of test sample, signal stabilization, etc. can be utilized with existing solid phase formats, e.g., flow-through pads, dipsticks, teststrips, etc.

A further aspect of the present invention is the use of a printable medium to define the capillary track as described below and as shown in Figures 4, 5, and 5a.

As depicted in Figure 4, the sides (25a) of the capillary track (18) are formed from a printable material, including, but not limited to ink or adhesive, that is insoluble, and preferably, that aids in adhering the first liquid occlusive layer (23) to a third liquid occlusive layer (26). The material is applied to either the first or third layer, and the sides are then capped by the application of the third or first laminate layer, respectively, thereby defining the capillary track (18).

The first (back laminate) layer (23) may be a flexible plastic or plastic-coated film supplied in either sheet or roll form. The film can also be chosen to have distinct properties such as opacity, biodegradability, etc., or it can be treated to have certain properties, such as hydrophobicity, hydrophillicity or selective biocompatability. The film may be selected from a variety of materials including, but not limited to, polyester, polycarbonate, and other film materials. In addition, the surface or a portion of the surface of the capillary track may be spot treated to create or enhance a desired property. For example, a portion of the capillary track may be

spot treated with a hydrophobic material to slow the rate of flow through that portion of the track. Suitable film materials include, but are not limited to, Mylar® film (DuPont, Wilmington, DE) and polyester films (Melinex® films; ICI Films, Wilmington, DE). Suitable film materials include materials having the following properties or characteristics:

Film Properties	Typical	Range
Thickness:	0.002 inch	0.0002 - 6.0 inches
Flexible:	30,500 psi tensile	any flexible or rigid material
Optical:	opaque white	transparent, opaque, reflective, metallic, or treated
Material:	polyester	any plastic, glass or metal or combination thereof

The second (core or sandwiched) layer (25a) is conveniently applied via printing techniques, such as screen printing methods which are well-known in the art. The second layer may be applied or deposited, however, by any suitable printing method capable of achieving the desired design tolerances for thickness, alignment, or geometric limitations of the capillary track. It will be appreciated by those skilled-in-the-art that the capillary track dimensions will be selected to accomplish the desired fluid delivery and timing characteristics which may differ between devices based upon the analyte of interest, the test sample used, incubation and reaction requirements, and other assay parameters. The second layer may be printed from an adhesive material, an ink, a dielectric material, or any material that is suitable for printing and for providing the desired thickness or height of the capillary track. The second layer may preferably be formed from an adhesive and more preferably a pressure sensitive adhesive. Pressure sensitive adhesive materials generally consist of a polymer formulation and are usually vinyl or acrylic based (e.g., UVC 8201, UVC 8200, ML 25184; Acheson Colloids, Port Huron, MI). Suitable pressure sensitive adhesives include, but are not limited to, materials having properties similar to UVC 8201 polyester film:

Adhesive Properties	Typical	Range
Solids Content:	100% solids	20% - 100%
Density:	8.48 lbs./gal.	5.4 - 13.4 lbs./gal.
Viscosity:	1700 - 2000 cps.	100 - 750,000 cps.
Color:	transparent	any
Cure:	U.V.	air dry, heat cure, U.V., solvent evaporation; crosslink
Material:	Urethane Acrylate	any -- acrylic, epoxy, vinyl, conductive, non-conductive, adhesive
Coverage:	1600 sq. ft./gal @ 1 mil	any -- to meet the desired film thickness
Adhesion (90 degree peel test):	2 lbs./in.	0.25 - 100 lbs./in.
Printed Film Thickness:	1 - 5 mils	0.2 - 10 mils

The printed material may be selected to have a suitable adhesiveness to laminate the top and bottom layers, as well as a suitable hydrophilic characteristic to promote the movement of fluid through the capillary track. Alternatively, the printed material may be selected to have a suitable hydrophobic characteristic to inhibit or prevent fluid flow within a portion of the capillary track, thereby controlling the rate of fluid flow through the device.

The pressure sensitive adhesive is applied in the reverse image of the capillary track, such that the printed area or areas define the thickness of the core layer (25a) and the non-printed area or areas form a gap between the first and second laminate layers, thereby defining the sides of the capillary track. The pressure sensitive adhesive may be applied using standard screen printing equipment (such as a flatbed screen printer from deHaart Inc, Burlington, MA). Typically, the second layer is applied in a single printing pass to a thickness ranging from 0.0002 inches to 0.010 inches. The layer, however, can be formed to have any desired thickness if accomplished in multiple passes. Usually, the layer will not exceed a final thickness of 0.100 inches.

Following application, the pressure sensitive adhesive is cured. For example, the pressure sensitive adhesive may be cured by means of ultraviolet radiation at about 200 watts per inch, or any other power rating that accomplishes crosslinking of the polymer to achieve the desired film properties such as thickness, adhesion and

tack. A release liner may be placed over the printed adhesive for handling purposes until device assembly, although assembly can take place immediately after cure. In conventional device construction methods involving a die cut film material with a two-sided adhesive, there are two layers of release liner to be removed prior to assembly, and assembly is more cumbersome. In the present invention, the core layer of printed pressure sensitive adhesive is applied directly to one side of the base or back film, thereby avoiding this handling step.

The third (base) layer (26) may be selected from the same film material as the first laminate layer. A different material may be used, however, to meet desired fluid flow characteristics or other properties pertinent to the design of the desired capillary track. A plastic or plastic-coated film, chosen for properties of opacity and wettability (e.g., plastic-coated paper board 150HT, Daubert, Dixon, IL; Vistex PC polyester film, Film Specialties, Inc., Whitehouse, NJ), is received in a roll and is applied to the printed core layer via standard web-laminating or processing techniques which are well-known in the art. Pressure is applied to bond the back and base layers to the core of pressure sensitive adhesive thereby forming the top and bottom of the capillary track. The base film may be slit, or die punched, or laser cut to produce fixture holes or other features of the desired design such as inlet and outlet ports for the capillary track. The third film layer, like the first, can be treated to have certain properties, such as hydrophobicity, hydrophillicity or selective biocompatability. Either the whole layer may be treated or at least that portion of the layer which forms the capillary track may be treated. Such treatment materials may also be advantageously applied by printing techniques.

In yet further embodiments, as depicted in Figures 5 and 5a, the second layer (25b) is made of a porous or liquid absorbent material which is selectively impregnated through its thickness with a substance, such as a water-repellent ink, to form an impregnated region (30) and a non-impregnated region (18). The core layer of porous material (25b) defines the thickness of the gap between the top (26) and bottom (23) laminate layers, and the impregnated region of the porous material defines the side walls of a porous capillary track (18). Thus, the non-impregnated region remains liquid absorbent, and the impregnated region is made liquid-occlusive, such that the non-impregnated region defines a solid (porous) capillary track for the passage of fluid via capillary action. Thus, the non-impregnated region, with inlet and outlet portions, serves as the means for directing the test solution through the device to the overlying porous material.

The porous second or core layer (25b) can be constructed using any suitable porous medium which typically has characteristics similar to the porous support materials. An exemplary porous medium is conventional filter paper (Whatman, UK; or Schleicher & Schuell 410, Keene, NH). The porous medium is generally printed

with a pressure sensitive adhesive or ink by means of equipment and printing techniques well-known in the art. The printed pattern defines the capillary track because the pressure sensitive adhesive or ink inhibits fluid-flow through the printed portions of the porous medium. As shown in Figure 5, the use of a printable, liquid-occlusive substance lacking adhesive properties requires the use of layers of a suitable adhesive material (28) on the surfaces between the porous layer (25b) and the base and back laminates (23) and (26), respectively. As shown in Figure 5a, the use of a pressure sensitive adhesive to print the porous medium has the added advantage of providing the adhesiveness for applying the base (23) or back laminate (26) layers directly to the porous material. The conventional method of device assembly involved cutting strips or pieces of the porous media and sandwiching that media between the back and base layers. With the present method, the roll of printed porous material is simply incorporated into the web process, thereby eliminating costly and complicated pick and place operations. Printable inks may be formulated to contain an assay reagent which is released from the printed layer as the test sample passes over the printed material. The filtering capacity of the porous medium in the capillary track may advantageously be used in the assay protocol. Moreover, different portions of the porous medium in the capillary track may be treated to modify the filter or transport characteristics of the medium. In addition, different portions of the porous medium in the capillary track may be treated to contain one or more assay reagent zones from which a reagent is released upon contacting the transported fluid.

The use of a printable medium to define the capillary track also provides for limitless design opportunities in terms of the geometric shape and variable thicknesses of the capillary track which characteristics may be used to control the rate at which the assay is performed. In addition, it simplifies device manufacture by eliminating the need for additional layer materials and material handling, while enhancing batch manufacturing procedures. The application of pressure sensitive adhesives and ink materials can be accomplished with any suitable method, including but not limited to, rotary or flatbed screen printing, flexographic printing, lithographic printing, letterpress printing, rotogravure printing, or ink jet printing. The devices may be printed in either a batch mode (one sheet at a time on either flexible or rigid film material) or in a roll with web processing methods (on primarily flexible film material). The printing processes can be accomplished with standard equipment. Exemplary processes are described in "The Printing Ink Manual" by R.H. Leach; "Handbook of Thick Film Technology" by P.J. Holmes and R. G. Loasby; or "Handbook of Thick Film Microcircuits" by Charles A Harper. Set up of the printer is within standard parameters and processes known in the art, or as described in the relevant instruction manual or in the above-mentioned texts.

Typical ink properties are also described, and for purposes of the present invention, the characteristics of suitable inks are similar to the characteristics of the printable pressure sensitive adhesives.

In yet another embodiment, as depicted in Figure 6, the sides of the capillary track are defined by a solid middle or core layer (25c) of film material which is laminated between a first layer (23) and a third layer (26) using two layers of a suitable adhesive material (28). The middle layer has a slot within its surface, partially or completely through the material, such that in combination with the first and/or third layers a capillary track (18) is formed. The adhesive material may also be die cut or printed to complement the slot in the middle layer such that the adhesive material does not form a part of the capillary track.

Figure 6a shows a further embodiment of a device of the invention having multiple, adherent, layers including a first or bottom wettable, but liquid-occlusive, layer (23), a second or middle liquid occlusive layer (24) parallel to and overlying the first layer (23), and a third or top layer (25d) of a porous or liquid absorbent material which is selectively impregnated through its thickness with a substance, such as a water-repellent ink, to form an impregnated region (30) and one or more non-impregnated regions or "islands" (32) positioned over the capillary track (18). Typically, as stated above, the second layer (24) is die cut or preformed to have a slot positioned through its thickness, thereby defining the walls of the capillary track (18) in conjunction with the first (23) and third (25d) layers. Furthermore, the device may also include an optional well-defining means (2) in the third layer (25d). The well-defining means is positioned such that the it defines an area for receiving the test sample, and it is in fluid flow communication with the capillary track. The bottom of the well may be formed from a corresponding circular portion of the first layer (23). Optionally, adhesive materials may be used to laminate the layers or, as decribed above, to form the capillary track. Thus, when the first, second and third layers are laminated together, a portion of each of the first and third layers serve as the floor and roof, respectively, of the capillary track with part of the walls of the slot of the second layer (24) defining the walls of the capillary track. The non-impregnated islands may be selectively pretreated with assay reagents in order to provide delivery of the reagents to the sample as the sample is drawn into the island by capillary action. The dimensions of the islands may be altered in order to provide differing and selective functions to the reagents placed therein. For example, selecting an island diameter which is smaller than the width of the capillary track promotes the rehydration of the reagent and subsequent transport of the reagent out of the island and into the sample as the fluid passes beyond the island. On the other hand, an island diameter larger than the width of the capillary track inhibits the tranport of reagents away from the island. Furthermore, each of the non-impregnated regions

may incorporate differing reagents as desired for the ultimate use of the device. For example, each of the islands (32) may incorporate differing concentrations of a labeled reagent and thereby provide signal development of a quantitiative nature. Alternatively, the regions may be treated with assay reagents which each interact with a different analyte such that more than one analyte may be detected in a single sample. In still a further alternative, each of the non-impregnated regions may be treated with assay reagents such that all reagents, e.g. such as may be necessary for a multi-step enzymatic assay, are present in the device prior to sample application.

In an optional modification of the device, the area of the porous support around the immobilized reagent may be at least partially compressed. Figure 7 depicts an embodiment wherein a nitrocellulose material (20) has been compressed in the area around the immobilized reagent site (22) to aid in the direction of fluid flow through the porous support and reaction site.

In another embodiment, the device may include an additional absorbent material in contact with the porous support. Figure 8 illustrates an embodiment representing a device (10) which includes an absorbent layer (40) surrounding the reaction site (22) on the porous support (20). The absorbent material serves to increase the liquid holding capacity of the device such that large test sample or reagent volumes may be used.

A preferred embodiment employs a housing which at least partially encloses a device of the invention for increased ease of use and handling. As shown in Figure 8a, the device (10) may employ a housing (52) which substantially encloses a device of the invention, e.g., as shown in Figures 1-3a and 7-8, and to which is affixed a drop-forming means (50), e.g., as shown in Figures 3a or 3b. After application of the test sample to the drop-former, the sample is delivered to the well-defining means (2) and flows via the capillary track (18) to the outlet port (16) and radially through the reaction site (22) on the porous support (20). Additional absorptive capacity can be supplied with the use of an absorbent pad (40) as described above. The housing may be made up of a non-wettable material, such as plastic, in more than one piece or section for ease of manufacturing and assembly. The pieces are then fastened to one another prior to use by techniques well known in the art. As will be readily apparent to those skilled in the art, additional variations of this embodiment may be designed to accomodate a wide variety of assays for a wide variety of analytes. Furthermore, as discussed above, the drop-forming means may be permanently affixed to the housing when in use or may be designed to be removable.

The main body of the devices of the present invention may be formed of a non-wettable material, such as a plastic material, or a wettable material, such as a porous material, wherein at least a portion has been rendered non-wettable. The capillary track typically has a total length of from about 0.5 to about 6.0 inches, preferably

from about 0.5 to about 2.0 inches. The structural arrangement of the device is generally designed such that about 50 to about 1000 microliters of test sample may be used to perform an assay. Preferably, the device is designed such that about 100 to 500 microliters of test sample is used. It will be appreciated by those skilled-in-the-art that the design will be optimized as needed to provide for the use of that amount of test sample required to perform the desired assay. The capillary track has a diameter suitable for the transport of such samples from the capillary track inlet to the capillary track outlet.

The test sample is introduced to the reaction zone in the porous support by means of the subsurface capillary track. The subsurface capillary track reduces the time required for a unit volume application of test sample to wet the same surface area of the porous support. Because the test sample and assay reagents are delivered directly to the reaction site, the test sample is not required to first pass through a non-reaction site portion of the porous support. This advantageous outcome is described by the D'Arcy Equation.

The D'Arcy Equation expresses flow rate as follows:

$$h^2=kt$$

where h = distance of chromatographic flow
k = flow constant
t = time

Where A_1 is equal to the area of a porous material in the shape of an elongated strip ($A_1 = h_1 \times w_1$), and A_2 is equal to the area of the porous support in the shape of a square or circle ($A_2 = h_2^2 \pi$) of the same thickness, the unit volume of test sample applied to each device format will flow as depicted in Figure 9. The linear uptake of a unit volume is substantially slower than that of radial uptake.

The present invention also provides for the ability to simultaneously perform multiple assays while utilizing a very small amount of test sample, for instance, a single drop. Such a device, in assembled form, has a plurality of capillary tracks. The device includes a sample application means which communicates with the inlet of the capillary tracks. The outlet of each track is in communication with a porous support. The individual porous supports are selectively impregnated with a specific binding member suitable for the detection of an analyte of interest. The number of tracks is not critical to the construction of the multiple track devices of the present invention. Alternatively, a single capillary track can include multiple outlets such that different outlets underlie different portions of a single porous support, wherein the different portions of that porous support contain immobilized binding members for different assays.

The assay devices of the present invention include a porous support in liquid communication with a capillary track, which support is typically positioned adjacent

to, and usually in direct contact with, the capillary track outlet. However, it has been discovered that the devices can include additional zones or layers between the capillary track and the porous support. Such zones may be used to further control the rate of flow between the capillary track and the porous support, may contain ancillary assay reagents or may be used to prevent or inhibit the transport of test sample interferents into the porous support.

Optionally, the flow rate per unit area of the capillary track can be gradually decreased along the general direction of flow by gradually increasing the space between the floor and the roof of the track along the direction of liquid flow. For example, flow may be decreased by gradually bowing the roof of the track upward and/or gradually bowing the floor of the track downward.

In an alternative embodiment of the present invention, a labeled reagent is positioned in the interior of the capillary track to form a reagent zone. When a test sample is introduced to one end of a capillary track, the test sample and labeled reagent combine to form a test solution. The test solution is transported through the track. The rate of liquid flow through the capillary track may be controlled, at least in part, by means of the porous support positioned at the distal end of the track. It has been found that a porous material, such as paper, may be utilized as the fluid flow control means to provide advantages in both manufacturing and performance over the coating of the track interior with water-soluble materials such as polyvinylpyrrolidone (PVP).

All types of specific binding assays can be accommodated with a device constructed in accordance with the present invention. Moreover, with the inclusion of all necessary assay reagents within the device itself, the assays may be made essentially self-performing once the test sample has been added to the device. For example, a soluble reagent (such as a labeled reagent) is dried within the capillary track during manufacture and is solublized upon contact with the test sample. In other instances, a labeled reagent can be contained by a soluble or porous matrix which is positioned within the capillary track itself. Suitable matrices which can release diffusive materials are well known in the art and include, but are not limited to, paper, sponge and glass fiber materials. In yet another embodiment, a reagent can be dispersed in a solution which is placed in the track.

The very small size of the reaction devices of the present invention advantageously allows for the rapid and convenient handling of a plurality of devices. A device can then be loaded into an automated apparatus which indexes and scans the individual reaction sites for the assay results and records this information for future access. The small dimensions of the device also provide for efficient use of sample and reagents. The present invention also provides for diagnostic kits employing the present devices in combination with containers of assay reagents which are not

incorporated within the device itself, for example, a test sample buffer solution or extraction solution.

EXAMPLES

The following examples are provided to further illustrate embodiments of the invention and should not be construed as a limitation on the scope of the invention.

EXAMPLE 1

A disposable device, as depicted in Figure 3, was constructed from a wettable base layer (23) (7 mil, hydromer-treated polyester; Film Specialties, Inc., Whitehouse, NJ), a die-cut adhesive core layer (24) (3 mil, double sided adhesive coated polyester film; Adhesives Research, Glen Rock, PA), a laser-machined, non-wettable, adhesive, laminate layer (26) (3 mil, single sided adhesive coated polyester film, Adhesive Research) and a microporous nitrocellulose pad (20) (5 micron pore size; Schleicher & Schuell, Keene, NH). The assembly of the wettable base, die cut core layer and laser-machined laminate form a subsurface capillary track with an inlet (2) at one end and a small outlet (16) at the other. The nitrocellulose membrane is laminated over the outlet to allow sample to dispense from the capillary track (18) into the membrane center thus avoiding linear chromatography from the membrane edge.

EXAMPLE 2

Human Chorionic Gonadotropin (hCG) Assay

A disposable device was constructed substantially in accordance with the description of Example 1. Anti-beta hCG antibody was applied to the center of the nitrocellulose pad in a "+" pattern with the two bars intersecting over the outlet in the laser-machined laminate. One of the bars included hCG to serve as a positive control for hCG-negative samples. Anti-alpha hCG antibody, and protein stabilizers, were absorbed on selenium particles (180 nM) to provide the labeled reagent for this sandwich assay.

Dry selenium conjugate pads were prepared by dipping glass fiber strips (Lydall, Inc., Rochester, NH) into a selenium conjugate solution and then passing the material through a drying tunnel. Circles (approximately 10.6 mm (0.420 inches) in diameter) were punched from the material and held in a molded drop forming means as shown in Figure 3a.

An hCG test sample (250 to 400 microliters) containing buffer or urine was applied to the assembly. A bolus of selenium conjugate was delivered to the track if the drop forming means was held above the track to allow a hanging drop to form. The drop fell onto the capillary inlet, filled the capillary track and was transported to the

nitrocellulose pad and through the capillary outlet directly beneath the center of the immobilized reagent "+". As the solution was radially transported in 360 degrees through and from the immobilized reagent site, a visible signal was formed at the reaction site in the form of a "+" if hCG was present in the sample, and in the form of a "-" if no hCG was present.

Strep A Assay

A disposable device was constructed substantially in accordance with the description of Example 1. Anti-Strep A antibody was applied to the center of the nitrocellulose pad in a "+" pattern with the two bars intersecting over the uptake hole in the laser-machined laminate. One of the bars included a protein containing the immunodeterminate recognized by the anti-Strep A antibody and served as a positive control for Strep A-negative samples. Anti-Strep A polyclonal antibodies, with protein stabilizers, were absorbed on selenium particles (180 nm) which served as the labeled reagent for this sandwich assay.

Dry selenium conjugate pads and drop forming means were constructed as described above. Upon application of the test sample, a signal developed at the reaction site in the form of a "+" if Strep A immunodeterminate was present in the sample, and in the form of a "-" if no Strep A immunodeterminate was present.

Results

Upon applying a test sample to the devices of the present invention, the average time for the sample to contact the nitrocellulose pad was five to ten seconds. Depending on the test sample hCG concentration, signal could be seen as a "+" in 30-40 seconds (250 mIU/ml) or between one and three minutes for test sample concentrations of 5-25 mIU/ml. Similar results were found for high and low levels of Strep A immunodeterminate in a test sample. It will be appreciated by those skilled-in-the-art that these reaction times may be further modified through reagent optimization. A novel and unexpected aspect of this technology is that the labeled reagent passing from the conjugate pad is concentrated in the first drop dispensed from the drop forming means into the capillary track. Typically, over 50% of the conjugate is concentrated in the first formed drop. Preferably, over 70% of the conjugate is concentrated in the first formed drop. In the most preferred form, over 90% of the conjugate is concentrated in the first formed drop. The immobilized reagent is then subjected to a bolus delivery of labeled reagent with the test sample, and as subsequent test sample passes through the test device unreacted labeled reagent is cleared from the reaction zone. The resulting signal was thus enhanced as the background field of the immobilized reaction site changed from pink to white and the immobilized signal remained red.

EXAMPLE 3

A disposable device was constructed from a wettable base (7 mil hydromer-treated polyester, Hydromer, Inc.), a die-cut adhesive core layer (3 mil, double sided adhesive coated polyester film (Adhesives Research) and a printed filter-paper top layer (S&S grade 410). A hole was punched in the printed part of the filter paper layer to serve as a sample entry means. The assembly of the wettable base, die-cut core layer and printed filter-paper top layer formed a subsurface capillary track with a sample uptake hole in the paper top layer where ink was not deposited. The printed pattern in the track allowed sample to fill the track to a predetermined point. The printed pattern in the top layer allowed solution to travel within the track and react with reagents that were deposited in the wettable, non-printed areas of the top paper layer.

EXAMPLE 4

Glucose Test

A glucose test device was constructed by using a disposable device as described in Example 3 and assay reagents for an enzymatic glucose determination. The color forming reagent was 4-chloronapthol which was spotted onto the filter paper as 25 mg/ml acetone solution and allowed to dry. Solutions of glucose oxidase and horseradish peroxidase (Sigma, Inc.; St. Louis, MO) in a phosphate buffer were then spotted on the filter paper in the wettable regions containing the dried 4-chloronapthol. Concentrations of glucose oxidase were chosen to allow the color formation reaction to proceed at different rates between each wettable region. The test was begun by adding a buffered solution containing glucose to the entry hole.

Results

Upon sample application, the fluid flowed via capillary action through all of the wettable regions and color formation began. Rates were monitored using a light transmission device. Each wettable area was monitored independently.

EXAMPLE 5

A disposable device and reagents, as described in Example 3 and 4, were used to make a glucose test device. The wettable base layer had a pattern of hydrophobic ink printed down the capillary track. Upon application of a buffered solution containing glucose, the track filled down to the hydrophobic ink section of the track. The device was attached to a rotating means (e.g., Dremel tool) through a hole in the center of the device. Upon applying centrifugal force to the device, the sample was forced past the hydrophobic track section and into a holding area of the track. Upon

release of the centrifugal force, the solution traveled up the track into the reagent area where the glucose-determining reactions began.

Blood separation was accomplished in the device by applying a whole blood sample to the sample uptake hole as described above for the buffered-glucose solution. Upon application of centrifugal force the blood cells separated out in the bottom of the track past the hydrophobic track area. With the release of the centrifugal force, plasma decanted away from the compacted cells and traveled up into the reagent portion of the test where the glucose-determining reactions began.

EXAMPLE 6

A disposable device is constructed by using a film material as the first or base layer. The film may also be used as the third or top layer. The top layer is cut by a laser to form two holes which provide the capillary inlet and outlet ports once the device is constructed. The bottom surface of the third layer is screen printed with an aqueous adhesive leaving an unprinted area between the two holes which defines the sides of the capillary track. The bottom surface of the third layer provides the top of the capillary track. The adhesive material is cured, and the base layer is placed over the printed material. A suitable amount of pressure is applied to adhere the base layer, thereby forming the bottom of the capillary track.

A porous support is positioned on the upper surface of the top layer substantially over the capillary track outlet such that the outlet is in fluid flow communication with the porous support. An assay reagent may be immobilized on the porous support before or after the support is attached to the film.

EXAMPLE 7

A disposable device is constructed substantially in accordance with the technique described in Example 6, with the exception that an adhesive is applied to both the first and third layers.

EXAMPLE 8

A disposable device is constructed substantially in accordance with the technique described in Example 6, with the exception that a two-sided adhesive material having suitable release liners and a slot to define the sides of the capillary track is applied to either the first or third layer to form a middle layer. For example, a release liner is removed from one side of the adhesive material and aligned over the base layer, and pressure is applied. The second release liner is then removed, the third layer is aligned over the adhesive, and pressure is applied to complete the construction of the capillary track.

EXAMPLE 9

Drop-formers are constructed as follows. Dry selenium conjugate pads are prepared as described in Example 2. 10.6 mm discs are cut from sheets of the following glass fiber materials: Nalgene® #281-9000 (Nalge Inc., Rochester, NY, USA); Ahlstrom #161; Whatman F300 (Whatman, Inc., Maidstone, Kent, Great Britain); H&V(DC, DD, DE, HA, KB series)(Hollingsworth & Vose, West Groton, MA, USA); and Lydall® (Lydall, Rochester, NH, USA). The discs are placed in molded drop forming means as shown in Figure 3b. One set of drop-formers is made using a single layer of Lydall® as described in Example 2. A second set is constructed of a first, or bottom, layer of Lydall® glass fiber as described in Example 2 and a second, or top, layer of a material listed above. The devices are fixed in place over inlet ports of capillary devices constructed substantially as shown in Example 2. Negative (no hCG) urine samples (200 to 400 µLiter) are placed on the top of each of the drop-formers and allowed to pass through to the respective inlet ports below. The results are viewed and show that devices utilizing the single layer device have inhibited development of control (-) signal as compared to devices utilizing a two layer drop-former. The best results are obtained using Nalgene® #281-9000.

EXAMPLE 10

Drop-formers are constructed as follows. Dry selenium conjugate pads are prepared as described in Example 2. 10.6 mm discs are cut from sheets of the following materials: absorbent paper (coffee filters, store brands); H&V (KD and 43-68G series); Tetko (9-7/2, 3/10/2. and 3-5/1); PD #075 (Whatman); Porex® (Porex Technologies); Ahlstrom (988 and 953); Fabray 110 (Stearns Technical Textiles Co., Cincinnatti, OH, USA); ZBF (#1 and #2) (ZBF, Rüschlikon, Switzerland); and Lydall®. The discs are placed in drop-formers substantially as described in Example 9. One set of drop-formers is made of a single layer of Lydall as described in Example 2. A second set is constructed as described in Example 9. A third set is constructed of a first, or bottom, layer of the material listed above, a second, or middle, layer of Lydall glass fiber as described in Example 2, and a third, or top, layer of a disc of Nalgene glass fiber (#281-9000). The devices are fixed in place over inlet ports of capillary devices constructed substantially as shown in Example 2. Negative (no hCG) urine samples (200 to 400 µLiter) are spiked with hCG at 25 mIU/mL and the samples applied to the top of the drop-formers. The results are viewed and show that as compared to a single Lydall pad or the two-layer drop-former of Example 9, the three layer drop-former improves positive (+) signals of urine samples spiked at 25 mIU/mL. The best results are obtained using absorbent paper as the bottom layer. It is believed that the third layer acts to slow the passage of the sample through the drop-former, thus allowing improved mixing of

test sample (hCG) and conjugate (anti-alpha hCG antibody-selenium microparticle) prior to passage of sample to the inlet port.

It will be appreciated by those skilled-in-the-art that the concepts of the present invention are applicable to various types of assay configurations, analytes, labels and device materials. The embodiments described herein are intended as examples, rather than as limitations, of assay devices using subsurface flow. Thus, the description of the invention is not intended to limit the invention to the particular embodiments disclosed, but it is intended to encompass all equivalents and subject matter within the scope of the invention as described above and as set forth in the following claims.

What is claimed is:

1. A device for determining the presence or amount of an analyte in a test sample, comprising:

a capillary track having an inlet and outlet, wherein the inlet receives test sample or test solution, and the outlet is in communication with and directs test sample or test solution to a porous support;

a drop-forming means positioned adjacent the capillary track such that a drop so formed will be delivered to the inlet;

wherein the capillary track is constructed from a porous material selectively impregnated with a fluid repellent printable substance wherein a non-impregnated region defines two sides of said capillary track, and wherein the porous material forms a core layer between a first and a second laminate layers which form a top and a bottom of the capillary track;

the porous support containing an immobilized reagent which directly or indirectly binds a labeled reagent in relation to the presence or amount of analyte in the test sample, wherein the outlet port is disposed beneath the porous support.

2. The device according to claim 1 wherein the labeled reagent is contained within the drop-forming means.

3. The device according to claim 1 wherein some or all of the assay reagents are contained within the drop-forming means.

4. The device according to claim 1 wherein the drop-forming means comprises multiple layers.

5. The device according to claim 4 wherein the multiple layers consist of a pre-filter layer, a middle layer and a bottom layer.

6. The device according to claim 5 wherein the pre-filter is glass fiber, the middle layer is glass fiber impregnated with a labeled reagent, and the bottom layer is absorbent paper.

7. A drop-forming means comprising one or more layers of porous materials wherein assay reagents are contained within the porous materials.

8. The drop-forming means according to claim 7 wherein a labeled reagent is contained within the porous materials.

9. The drop-forming means according to claim 7 having two porous materials.

10. The drop-forming means according to claim 7 having a top porous material, a middle porous material and a bottom porous material.

11. The drop-forming means according to claim 10 wherein the top porous material is glass fiber, the middle porous material is glass fiber impregnated with labeled reagent, and the bottom porous material is absorbent paper.

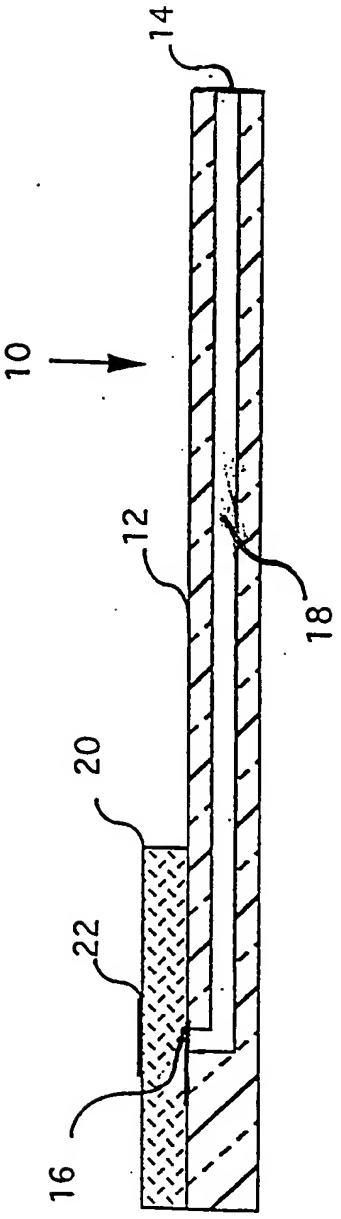

Figure 1

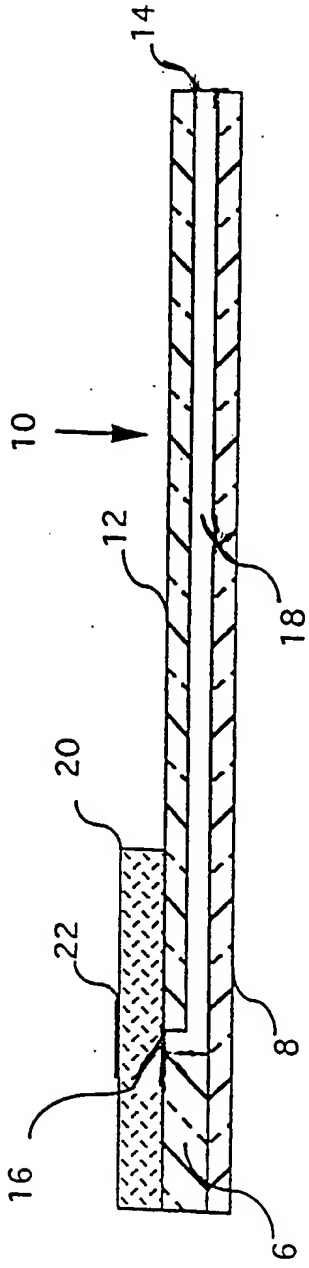

Figure 2

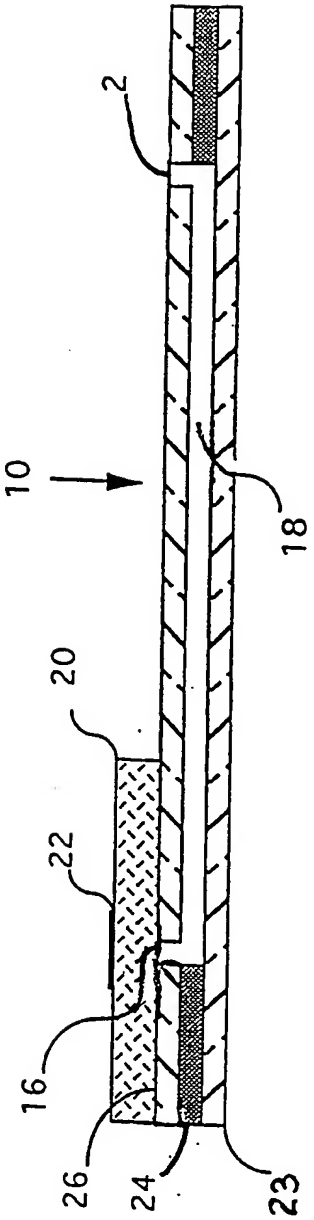

Figure 3

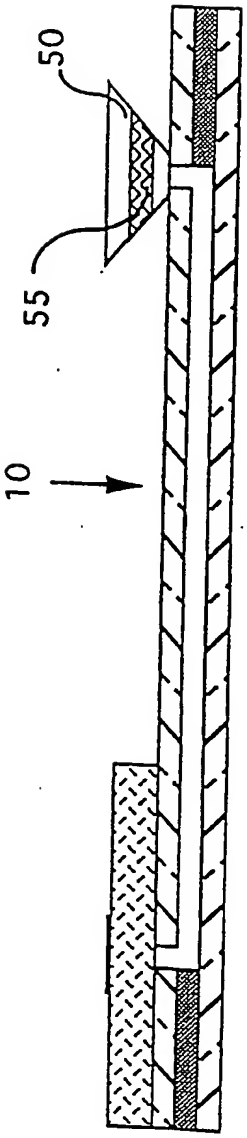

Figure 3a

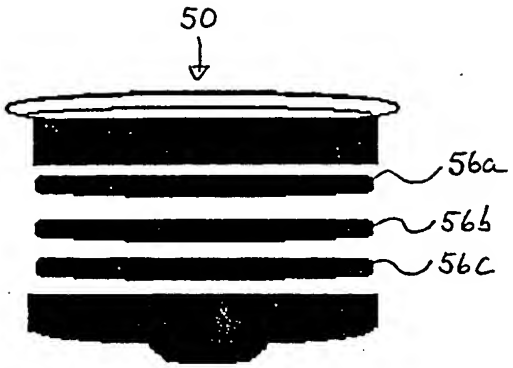

Figure 3b

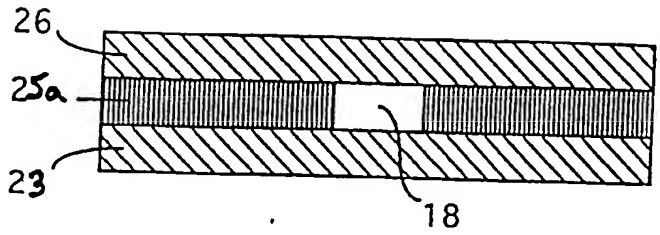

Figure 4

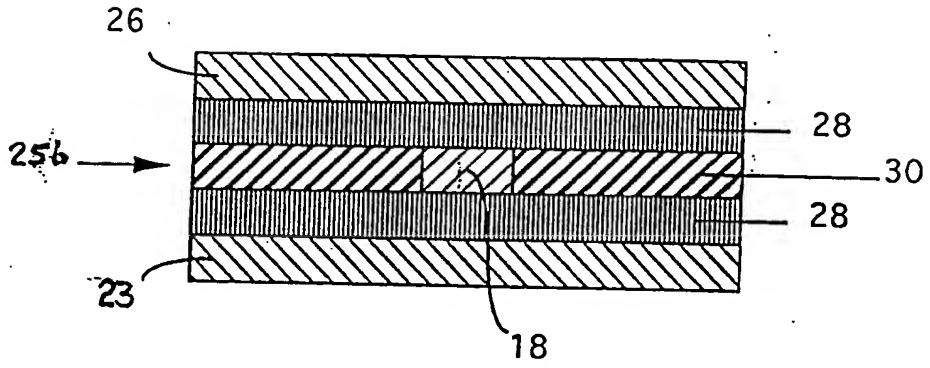

Figure 5

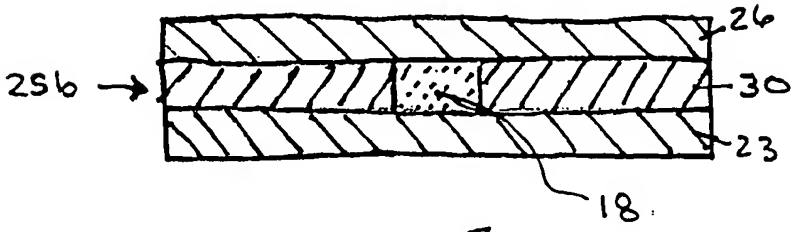

Figure 5a

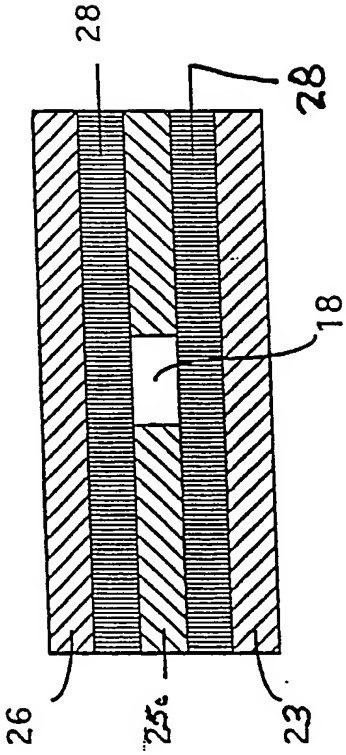

Figure 6

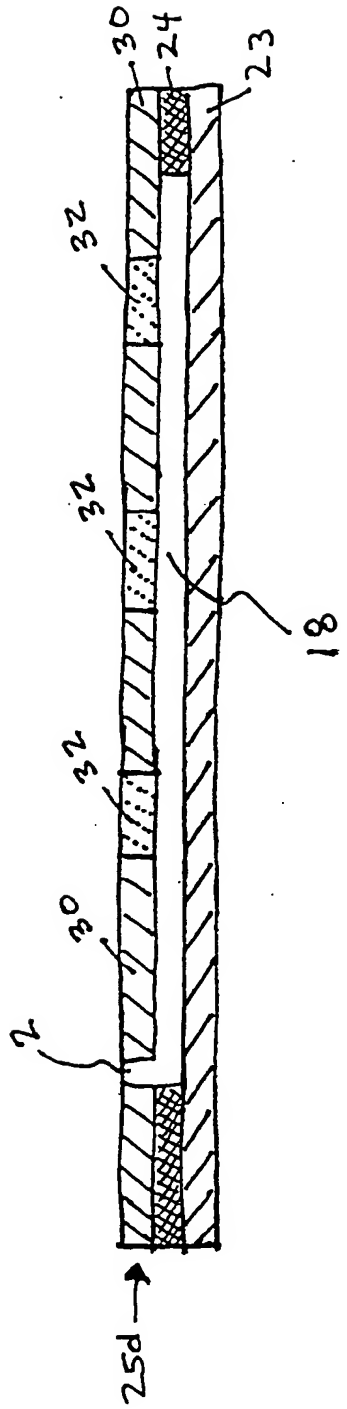

Figure 6a

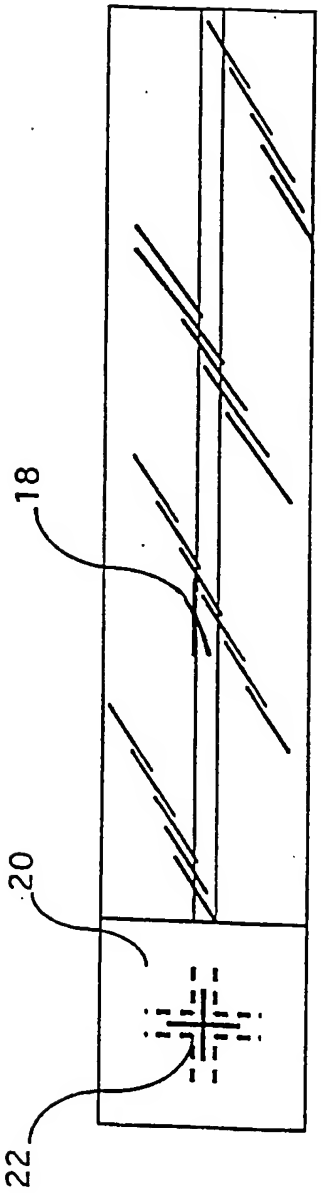

Figure 7

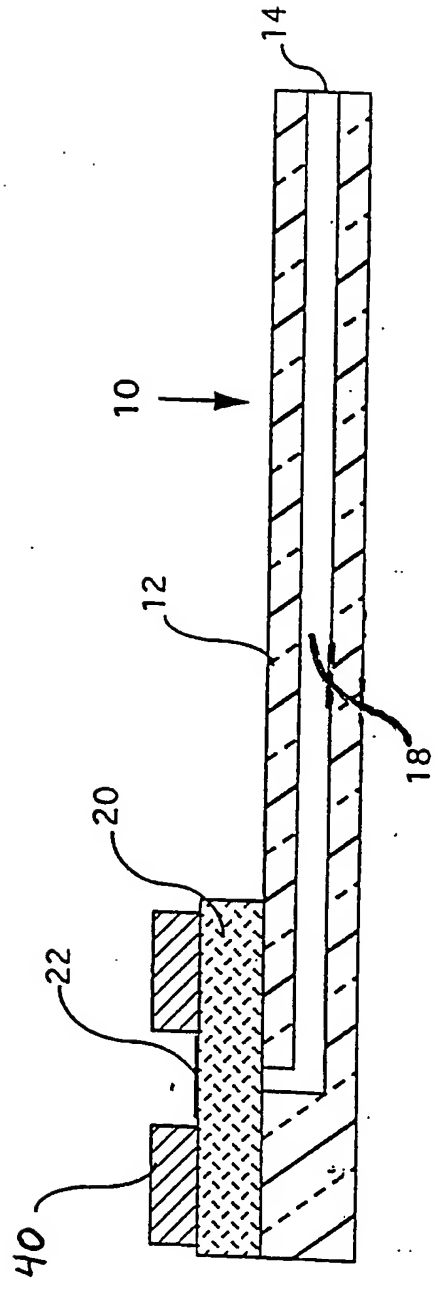

Figure 8

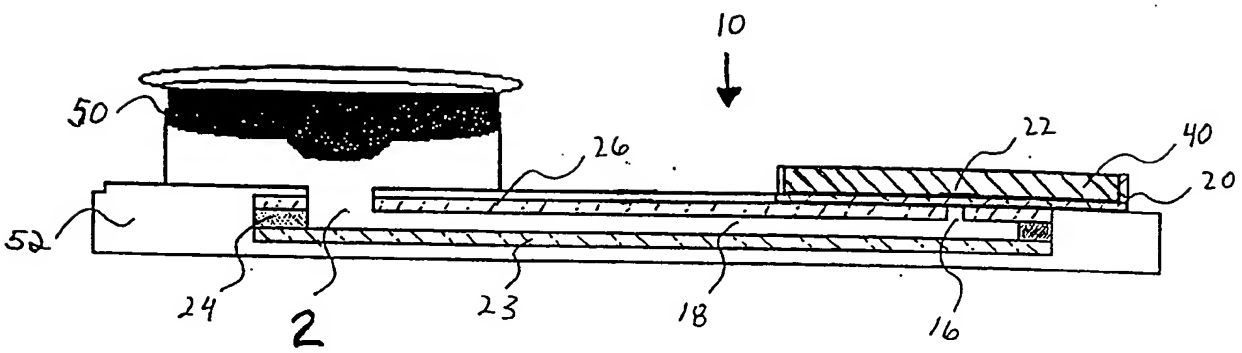

Figure 8a

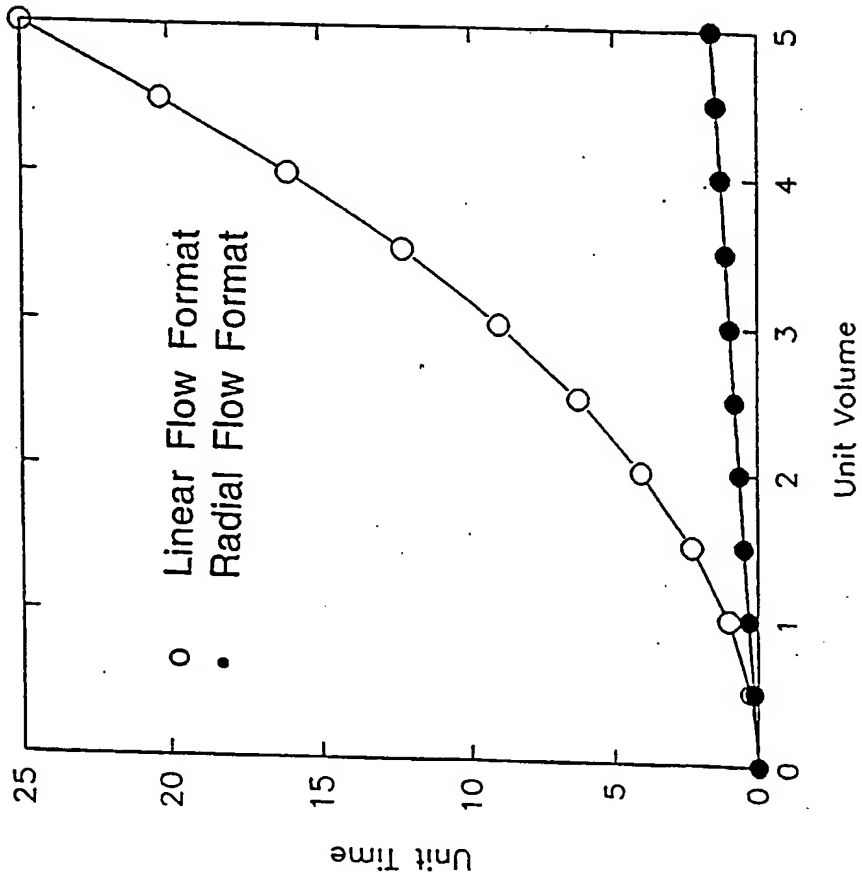
Linear Flow Format
Radial Flow Format
Unit Time
Unit Volume
0
5
10
15
20
25
0
1
2
3
4
5

Figure 9

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 94/14510

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L3/00 G01N33/52 G01N33/543 B01L3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,94 09366 (ABBOTT) 28 April 1994 see page 11, line 11 - page 12, line 19; figure 3A see page 15, line 30 - page 16, line 15; figure 5 see page 19, line 31 - line 33	1-11
Y	WO,A,92 08972 (ABBOTT) 29 May 1992 see page 9, last paragraph - page 10, paragraph 1 see page 13, last paragraph - page 14, paragraph 1	1
Y	WO,A,93 20939 (BIOTRACK) 28 October 1993 see page 20, line 19 - page 21, line 5; figures 2,3A,5 see page 28, line 8 - page 29, line 8; figures 10,11	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April 1995	24.05.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Hocquet, A

Form PCT ISA 210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/14510

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 010 456 (EASTMAN KODAK) 30 April 1980 see page 7, line 15 - page 9, line 5; claims 2,8; figures 4,5	1
X	EP,A,0 398 739 (MONOCLONAL ANTIBODIES) 22 November 1990 see page 3, line 41 - line 46; figure 1 see page 5, line 6 - line 12 see page 5, line 25 - line 55 see page 6, line 24 - line 26 see page 7, line 29 - line 35; table 2	7,8
Y A	WO,A,92 07655 (HYPOGUARD LTD) 14 May 1992 see page 6, line 31 - page 7, line 17; claims 14,15; figure 1	7-11 1
Y A	US,A,4 933 092 (ABBOTT) 12 June 1990 see column 3, line 25 - line 40; figures see column 2, line 3 - line 31 see column 6, line 19 - line 55 see column 7, line 7 - line 45 see column 7, line 64 - line 8	7-11 2-6

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Inter nal Application No
PCT/US 94/14510

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 94/14510

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